RESEARCH ARTICLE

The Metal Effect on Self-Assembling of Oxalamide Gelators Explored by Mass Spectrometry and DFT Calculations

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Abstract. Gels formed by self-assembly of small organic molecules are of wide interest as dynamic soft materials with numerous possible applications, especially in terms of nanotechnology for functional and responsive biomaterials, biosensors, and nanowires. Four bis-oxalamides were chosen to show if electrospray ionization mass spectrometry (ESI-MS) could be used as a prediction of a good gelator and also to shed light on the gelation processes. By inspecting the gelation of several solvent, we showed that bis(amino acid)oxalamide **1** proved to be the most efficient, also being able of forming the largest observable assemblies in the gas phase. The formation of singly charged assemblies holding from one up to six monomer units is the outcome of the strong intermolecular H-bonds, particularly among terminal

carboxyl groups. The variation of solvents from polar aprotic towards polar protic did not have any significant effects on the size of the assemblies. The addition of a salt such as NaOAc or Mg(OAc)₂, depending on the concentration, altered the assembling. Computational analysis at the DFT level aided in the interpretation of the observed trends and revealed that individual gelator molecules spontaneously assemble to higher aggregates, but the presence of the Na⁺ cation disrupts any gelator organization since it becomes significantly more favorable for gelator molecules to bind Na⁺ cations up to the 3:1 ratio than to self-assemble, being fully in line with experimental observations reported here.

Keywords: Oxalamide gelators, Self-assembly, ESI-MS, DFT

Received: 16 May 2017/Revised: 2 October 2017/Accepted: 10 October 2017/Published Online: 30 October 2017

Introduction

Individual molecules can self-assemble into oligomers as a consequence of molecular recognition interactions between

the building blocks [1]. These oligomers subsequently grow to form fibrils by the same process and, in many cases, the fibrils then bundle together to form fibers. These fibers subsequently form extended networks, which are capable of supporting the gel [2].

To comprehend gelation induced by small organic molecules, it is of utmost importance to reveal the relationship between the gelator structure, gelled solvent properties, and the organization in fibrous supramolecular aggregates constituting the three-dimensional gel network [3]. Precise knowledge of this relationship would allow a reliable and target

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s13361-017-1834-5>) contains supplementary material, which is available to authorized users.

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design of a specific gelator for a liquid with selected properties as well as the preparation of gels suitable for various applications, especially in terms of nanotechnology for functional and responsive biomaterials, biosensors, and nanowires [4].

Oxalamides, being simple mono-substituted diamides of oxalic acid, represent construction units with a high in-plane hydrogen bonding potential; they are self-complementary, capable of unidirectional hydrogen bonding and, hence, ideally suited for the construction of self-assembled supramolecular systems of fibrous morphology. Our group has long-term experience in design, synthesis, and characterization of low molecular weight gelators, especially bis(amino acid)oxalamides [5–9]. They exhibit ambidextrous gelation properties, being capable of forming gels with apolar and also highly polar solvent systems and tend to organize either into bilayers or inverse bilayers in hydrogel or organic solvent gel assemblies, respectively. It is worth noting that the bulk solvent is immobilized as a consequence of capillary forces, with individual solvent molecules actually retaining their mobility within the gel on the molecular scale. ^1H NMR and FTIR studies of gels revealed the importance of the equilibrium between the assembled network and smaller dissolved gelator assemblies [10]. The organization in gel assemblies deduced from spectroscopic structural studies are in certain cases closely related to the organizations found in the crystal structures of selected gelators, confirming similar organizations in gel assemblies and in the solid state. Mixing of the constitutionally different bis(amino acid) and bis(amino alcohol)oxalamide gelators resulted, in some cases, in highly improved gelation efficiency denoted as synergic gelation effect (SGE) [11], being also highly dependent on the stereochemistry of the component gelators. Applications of oxalamide units in crystal- and bio-engineering may open new perspectives in the utilization of these systems as powerful and versatile organizational and directional element for the future construction of novel abiotic and biotic supramolecular systems.

The commonly used methods for the characterization of supramolecular gels are nuclear magnetic resonance spectroscopy, computational molecular simulations, X-ray and microscopy techniques, dynamic light scattering, thermal analysis, and rheology [12]. In addition to the commonly used methods mentioned above, other methods such as mass spectrometry have been sometimes used for the characterization of supramolecular gels. Thus, MacLachlan and coworkers used electrospray ionization mass spectrometry (ESI-MS) to get a snapshot of large aggregates of zinc salophen complexes in solution, which provided evidence for the formation of metal-

containing gel [13]. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) is another especially useful method for the detection of organometallic gelators [14, 15].

Electrospray ionization [16, 17], which is a soft ionization technique, has emerged in the past decade as an indispensable tool for analyzing supramolecular assemblies that contain non-covalent bonds [18–21]. The advantage of ESI is that it keeps the internal energy of the ions low and thus not only suppresses extensive fragmentation but also makes the intact ionization of non-covalent assemblies feasible. Moreover, the analyte can be ionized from almost any suitable solution, provided that a charge is present in the complex or can be delivered to it during ionization. A factor that governs whether tandem MS of non-covalent complexes can yield any structural information is the relative stability of the *gas phase* complex, which promotes intrinsic organization of the individual gelator molecules not influenced by the presence of any solvent or counterions.

The motivation for this work was to explore the difference in the gas phase self-organization as an indicator of molecular recognition interactions between the oxalamide building blocks. The physical linking could be observed due to the non-covalent supramolecular interactions. Beside, an organogelation process depends on the gelator–solvent pair. It is well established that this choice has a major importance in the search for specific properties of the material. Even though a lot of attention has been paid to the gelators, the solvent is usually chosen by a trial-error approach. Prediction of gelation potential of a given molecule might seem possible by investigation of its propensity towards chemical or physical intermolecular interactions; however, no generalizations are so far possible. In this paper, we highlight that the preserved self-organization observed in the gas phase via ESI-MS could possibly be an indication of good gelation properties. Namely, the self-assembling of compounds **1–4** (Figure 1) dissolved in protic and aprotic polar solvents and in the mixtures with non polar but very good gelling solvents was examined and the most interesting results are presented. The disruption of the gels with sodium and magnesium ions was examined experimentally and corroborated computationally.

Experimental

General

The solvents used for the spectroscopic measurements were HPLC or spectroscopic grade (Sigma-Aldrich Chemie GmbH,

Figure 1. Structure of studied bis(amino acid/ester/alcohol)oxalamide gelators **1–4**

Steinheim, Germany) and were used without further purification. Salts $\text{Mg}(\text{OAc})_2$ and NaOAc were purchased (Sigma-Aldrich Chemie GmbH, Steinheim, Germany; Merck AG, Darmstadt, Germany) and were used without further purification.

Synthesis

Compounds **1–4** (Figure 1) were prepared in our laboratory according to previously described methods [5, 7, 22].

Instrumentation

Self-assembling of compounds **1–4** (Figure 1) was studied by ESI, using the ion trap for MS^n studies, and triple-quadrupole for ESI-MS/MS studies. The spectra were obtained at the same concentrations and conditions in both positive ion (ES^+) and negative ion (ES^-) modes. Certain fragmentations occurred at higher collision energies. The fragmentation pathways for all analyzed compounds were proposed based on MS/MS and MS^n spectra of protonated molecular ions $[\text{M}+\text{H}]^+$ or deprotonated molecular ions $[\text{M}-\text{H}]^-$ as well as sodium adducts.

1. The mass spectral data were acquired using an Agilent 6420 Triple Quad mass spectrometer equipped with an electrospray ionization interface operated in the ES^+ and ES^- modes (Agilent Technologies, Palo Alto, CA, USA). The samples were prepared in water, D_2O , methanol, methanol- d_4 , ethanol, ethanol- d_6 , acetonitrile, acetonitrile- d_3 with and without NaOAc , or $\text{Mg}(\text{OAc})_2$ to a concentration of about 0.05 mg/mL and directly injected. The infusion into the mass spectrometer was performed at a flow rate of 3 $\mu\text{L}/\text{min}$. Nitrogen was used as an auxiliary and sheath gas. The spray voltage was set at 4.5 kV. The capillary temperature was 150–300 $^\circ\text{C}$, and the voltage range of the collision cell was 80–180 V. The full mass spectra were acquired over the mass range m/z 10–2000. For data acquisition and analysis, Mass Hunter software (Agilent Technologies, Inc. 2006–007) was used. A parent ion window of typically 4 u (i.e., parent mass ± 2 u) was chosen to perform further MS/MS experiments.
2. The ESI- MS^n ($n > 2$, a parent ion window of typically 2 u (i.e., parent mass ± 1 amu)) experiments were recorded using an amaZon ETD mass spectrometer (Bruker Daltonik, Bremen, Germany) equipped with the standard ESI ion source (the nebulizer pressure: 8 psi; the drying gas flow rate: 5 L/min; the drying gas temperature: 250 $^\circ\text{C}$). The mass spectrometer was operated in the positive and negative polarity modes, and the potential on the capillary cap was ± 4500 V. Helium was used as a collision gas. The samples were prepared in water, D_2O , methanol, methanol- d_4 , ethanol, ethanol- d_6 , acetonitrile, acetonitrile- d_3 with and without NaOAc or $\text{Mg}(\text{OAc})_2$ to a concentration of about 0.5×10^{-6} mol/dm³ and injected into the ESI source of the mass spectrometer by a syringe pump at a flow rate of 1 $\mu\text{L}/\text{min}$.

Computational Details

As a good compromise between accuracy and the computational feasibility, all geometries were optimized by the very efficient M06-2X/6-31+G(d) method in the gas phase with thermal Gibbs free energy corrections extracted from the corresponding frequency calculations without the scaling factors. The final single-point energies were attained with a highly flexible 6-311++G(d,p) basis set giving rise to the M06-2X/6-311++G(d,p)/M06-2X/6-31+G(d) model used here. In this way all of the reported computational data correspond to the gas-phase Gibbs free energies at a temperature of 298.15 K and a pressure of 1 atm. The choice of such a computational setup was prompted by our recent success in modeling the properties of gelator molecules [23] and metal binding affinities [24], as well as correctly reproducing thermodynamic and kinetic parameters of organic [25, 26] and enzymatic [27] reactions. In order to circumvent problems with the flexibility of the investigated systems, we tried many different conformations in each studied case and report here the results corresponding to the most stable structures. All calculations were performed by using the Gaussian 09 program package [28].

Results and Discussion

A brief overview of the MS profile of the model compounds **1–4** is presented first, followed by the elucidation of the new species formed attributable to their non-covalent interactions. A detailed study was performed in the gas phase using CID (collision induced dissociation) experiments (ESI-MS/MS) and by the gas-phase density functional theory (DFT) calculations.

Bis(amino acid)oxalamide gelators or bis(amino alcohol)oxalamide are constructed from oxalo retro bipeptides. The core oxalyl (-CO-CO-) unit in this molecule can be considered as a dimerization element that links two identical amino acids into a novel C2 symmetric retro-bispeptide wherein the oxalamido (-NH-CO-CO-NH-) unit is locked in a perfect *trans* conformation [29]. The pseudo C₅ type intramolecular hydrogen bonding motif is responsible for the formation of highly organized self-assemblies [30, 31].

Bis(amino acid)-, **1**, bis(amino ester)-, **2**, bis(amino amide)-, **3**, and bis(amino alcohol)-, **4**, oxalamide gelators represent a class of versatile gelators, the gelation ability of which is a consequence of both strong and directional intermolecular hydrogen bonding provided by oxalamide units, and a lack of molecular symmetry attributable to the presence of two chiral centers. Even though ESI is a mild technique and the non-covalent interactions such as hydrogen bonding or electrostatic interactions could be transferred into the gas phase of the instrument, we did not neglect the fact that during the transition from condensed phase into high vacuum, various properties of the non-covalent bonds – strength, directionality, and geometry – may significantly change. Any interaction that competes with the solvent could, upon evaporation, either increase in strength (like hydrogen bonds or electrostatic interactions) or decrease in strength (like hydrophobic interactions). Taking all of this

into account, we believe that the observed results in the gas phase could be related to the gelation tendency of four compounds presently studied.

Terminal Group Effect on Self-Organization in Gas Phase

The first part of the study is focused on the comparison of the terminal groups of four oxalamides: COOH, CH₂OH, COOCH₃, and CONH₂, and their influence towards self-assembling. Bis(amino acid)oxalamide **1** proved to be the best of all four studied gelators by gelling all studied solvents, (Supplementary Information Table S1). Transfer to gas phase and the detection of created self-assemblies of **1** was examined in different solvents via ESI-MS. Several intensive signals were observed in full scan spectra of **1** assigned as hydrogen or sodium adducts or various sizes of self-assembled units together, Figure 2. The formation of singly charged assemblies holding from one up to six (possibly even more) monomer units was the outcome of the strong intermolecular H-bonds, Table 1. The hypothesis was to check if the variation of the

specific solvation driven by protic capabilities of the solvent could obstruct the formation of hydrogen bonds between the gelator building blocks. The variation of the solvent: acetonitrile (polar aprotic) towards methanol, ethanol, or water (polar protic) did not have any significant effects on the size of the observable assemblies, pointing out that intermolecular hydrogen bonding overcome specific solvation effects as well as other non-covalent interactions. This observation confirms the fact that the bulk solvent is immobilized as a consequence of capillary forces, with individual solvent molecules actually retaining their mobility within the gel on the molecular scale. It also agrees with their ambidextrous gelation properties: being capable of forming gels with apolar and also highly polar solvent systems and tending to organize either into bilayers or inverse bilayers in hydrogel or organic solvent gel assemblies, respectively.

The analysis of the collision induced fragmentation spectra of major observed signals pointed out that the decarboxylation of H-adduct assemblies occurred very easily in both modes (ES^{+/−}) showing the strength of the intermolecular H-bonds. It was confirmed by the presence of the fragments at *m/z* 271 or 226 in ES⁺ assigned as the mono- and didecarboxylated monomer of acid **1** (Supplementary Information Figures S2 and S3) or *m/z* 541 and 585 in ES[−] assigned as the mono- and didecarboxylated dimer of acid **1**. Sodium adducts had higher prevalence (values shown in *italic* in Table 1) and did not show any decarboxylation products; alternatively, the departure of the monomer units was observed, which was the dominating fragmentation path in all sodium adducts studied. There was also the difference in stability of the observed adducts. Namely, the fragmentation of the protonated species such as one at *m/z* 428 decarboxylated already at CE 5eV, whereas the sodium adducts did not decarboxylate easily (CE ≥15eV).

Three other low molecular weight gelators (LMWG) (**2**, **3**, and **4**) were examined in order to compare their assembling regarding their structural differences: the variation was in terminal groups: COOCH₃, CONH₂, and CH₂OH. The evaluation of the self organization of compounds demonstrated that bis(amino ester) oxalamide, **2**, and bis(amino alcohol) oxalamide, **3**, formed dimers as the largest species, Figure 3, whereas bis(amino acid) oxalamide, **1** and bis(aminoamide) oxalamide, **4** self organized in larger aggregates (six and more monomers in aggregates), Figure 4 and Table 1.

Alcohol **3** and ester **2** have oxalamide hydrogens capable of forming intermolecular H-bonds analog to acid **1** or amide **4** but it seems that the additional hydrogen from either the terminal carboxyl group or amide group was essential for the formation of a whole new array of aggregates observable in the gas phase in regard to -OH or -OCH₃ groups, Figure 5, as will be confirmed by the computational analysis presented later. The observation of large self-assemblies in the gas phase goes well with the fact that the additional H-bonds were essential for the in source fragmentation survival. Those bonds were responsible obviously for their excellent gelling capabilities too (Supplementary Information Table S1). Additionally, the X-ray data about the self-assembly in the solid phase showed that acid

Figure 2. Full scan ES⁺ spectrum for bis(amino acid)oxalamide **1** dissolved in water about 10^{−5}M; divided to two *m/z* regions (a) 200–1000; (b) 1200–2000 for clarity

Table 1. Species Observed for Self Organized Bis(amino acid) Oxalamide, **1** Dissolved in Acetonitrile (Supplementary info Figure S1a–f for the Spectra in Methanol, Ethanol, Water, Water/NaOAc), and Bis(Amino Amid)oxalamide, **4** in Water, Scanned in ES⁺ Mode

Monomer		Dimer		Trimer		Tetramer		Pentamer		Heksamer	
Species	<i>m/z</i>	Species	<i>m/z</i>	Species	<i>m/z</i>	Species	<i>m/z</i>	Species	<i>m/z</i>	Species	<i>m/z</i>
[1H] ⁺	317	[1 ₂ H] ⁺	633	[1 ₃ H] ⁺	949	[1 ₄ H] ⁺	1264	[1 ₅ H] ⁺	-	[1 ₆ H] ⁺	1896
[1Na] ⁺	339	[1 ₂ Na] ⁺	655	[1 ₃ Na] ⁺	971	[1 ₄ Na] ⁺	1287	[1 ₅ Na] ⁺	1602	[1 ₆ Na] ⁺	1919
[1Na ₂] ⁺	361	[1 ₂ (H ₂ O)] ⁺	651	[1 ₃ (H ₂ O)] ⁺	966	[1 ₄ Na ₂] ⁺	1309	[1 ₅ Na ₂] ⁺	1625	[1 ₆ Na ₂] ⁺	1941
		[1 ₂ (H ₂ O) ₂] ⁺	669	[1 ₃ (H ₂ O) ₂] ⁺	984	[1 ₄ Na ₃] ⁺	1331	[1 ₅ Na ₃] ⁺	1647	[1 ₆ Na ₃] ⁺	1963
		[1 ₂ Na ₂] ⁺	677	[1 ₃ Na ₂] ⁺	995	[1 ₄ Na ₄] ⁺	1353	[1 ₅ Na ₄] ⁺	1670	[1 ₆ Na ₄] ⁺	1986
		[1 ₂ Na ₃] ⁺	699	[1 ₃ Na ₃] ⁺	1015	[1 ₄ K] ⁺	1303	[1 ₅ Na ₅] ⁺	1693		
		[1 ₂ K] ⁺	671	[1 ₃ K] ⁺	987	[1 ₄ K ₂] ⁺	1342	[1 ₅ Na ₆] ⁺	1715		
[4H] ⁺	315	[4 ₂ H] ⁺	629	[4 ₃ H] ⁺	943	[4 ₄ H] ⁺	1257	[4 ₅ H] ⁺	1571	[4 ₆ H] ⁺	1885
[4Na] ⁺	337	[4 ₂ Na] ⁺	651	[4 ₃ Na] ⁺	965	[4 ₄ Na] ⁺	1279	[4 ₅ Na] ⁺	1593	[4 ₆ Na] ⁺	1907
[4Na (H ₃ O)] ²⁺	-	[4 ₂ Na (H ₃ O)] ²⁺	334	[4 ₃ Na (H ₃ O)] ²⁺	491	[4 ₄ Na (H ₃ O)] ²⁺	648	[4 ₅ Na (H ₃ O)] ²⁺	805	[4 ₆ Na (H ₃ O)] ²⁺	962

formed the β-network of bis(2-aminopropionic acid)oxalamide structure, whereas ester or alcohol did not [32]. Another interesting observation was a higher abundance for the tetramer and pentamer, the doubly charged assemblies of **4** most likely due to better ionization potential of the amide versus acid.

H/D Exchange

H/D exchange experiments in solutions were performed in order to further confirm the observations regarding the type of the non-covalent interactions between the units in the gas phase. Hydrogen exchange results confirmed that the building blocks are connected via intermolecular hydrogen bonds.

Bis(amino acid)oxalamide **1** was dissolved in deuterated solvents. Exchangeable protons in monomer or in the assemblies formed due to the electrostatic interactions were completely replaced. On the other hand, self-assembled dimers, trimers, etc. connected via intermolecular hydrogen bonds exchanged amide protons slower confirming that it was costly and required more energy and time (Supplementary Information Figure S5), since several bonds had to be broken and formed: the intramolecular H-bond, N–H–O, and O–D

from solvent. This is further corroborated by the computational analysis, which, at the M06-2X/6-31+G(d) level of theory, revealed that the free energy required to deprotonate the amide N–H group in **1** is 5.5 kcal mol^{−1} higher than to deprotonate the carboxyl COOH group in the monomer, whereas it is 10.5 kcal mol^{−1} higher in the corresponding dimers. This makes it reasonable to expect that this trend will be maintained even in the higher aggregates, thereby supporting experimental findings. We also observed that the addition of Mg²⁺ salt altered the H/D exchange of the shielded hydrogen more than Na⁺, Figure 6.

Disruption of Gels

The ability of oxalamide derivatives (**1–4**) to form supramolecular gels by means of extended hydrogen bonded chains has given rise to a rich field of synthetically and externally tunable soft materials, which has been the subject of intense investigation in recent years [5–9, 23]. Addition of hydrochloric acid, various metal salts, or ions to the gels significantly affects their gelation properties. Recently, Steed reported several examples affecting and tuning mechanical properties of urea supramolecular gels by metal addition, the properties of which can be

Figure 3. Full scan ES⁺ mass spectra for **2** and **3** dissolved in water, in concentration of about 10^{−5}M

Figure 4. Full scan ES^+ mass spectrum for **4** dissolved in water, in concentration of about 10^{-5} M. Insert shows the signals of the doubly charged species

changed by the coordination metal ion (anion-tuning) or halogen bonding to trigger supramolecular gel formation [33–35].

The addition of salts such as NaOAc or $\text{Mg}(\text{OAc})_2$, depending on the concentration, altered the assembling. Together with the singly charged species, at low concentration of Mg^{2+} or Na^+ doubly charged larger assemblies were observed (Supplementary Information Figure S4). When bis(amino acid)oxalamide **1** was dissolved in aqueous solutions with higher concentrations

of NaOAc or $\text{Mg}(\text{OAc})_2$ salts, the formation of larger assemblies failed, being in agreement with the observation in condensed phase that the salt disrupts the gel formation (Supplementary Information Figure S5). This feature will be rationalized by the computational analysis presented later.

The formation of the salt bridges (a combination of two non-covalent interactions: hydrogen bonding and electrostatic interactions) is possible under favorable circumstances. It seems that the salt bridges form between the carboxylic anions and sodium ions in larger assemblies preventing the low energy decarboxylation observed in non-sodium adducts. We know that the addition of sodium ions prevents the gel formation and the already formed gels get destroyed simply by shaking from the solution experiments, as revealed here through the computational analysis. The capability for binding of three sodium ions per monomer unit of **1** was observed (Supplementary Information Figure S6 a and b). Our calculations show that the most stable of such structures involves *trans* gelator molecule with two Na^+ cations serving as counterions for the carboxyl group at Na–O distances between 2.18–2.29 Å, whereas the third Na^+ ion is bound to one of the carbonyl atoms at 2.24 Å. In the *cis* analog, two Na^+ cations are located in the same way in the vicinity of the carboxyl groups, whereas the third Na^+ ion binds both carbonyl oxygens on the other side of the molecule at the Na–O distance of 2.20 Å. Still, this is not enough to make the *cis* analog a dominant conformation, which is 1.8 kcal mol^{-1} less stable than the *trans* counterpart.

There were no larger than four units assembled together at higher concentrations of sodium ions, suggesting that the electrostatic interaction dominated over the hydrogen bonding, e.g., the affinity of C=O toward sodium ion was greater than towards hydrogen of the other gelator molecule. Binding of the

Figure 5. Cartoon showing intramolecular H-bonds vital for the networking and assembling of gelators studied here

Figure 6. Overlaid mode of stretched out ES^+ spectra (monomer, dimer, and trimer) of compound **1** dissolved in ethanol, ethanol-d6, with and without Mg^{2+} or Na^+ in total concentration of $c = 3 \times 10^{-5} \text{ mol dm}^{-3}$

third sodium ion disrupts the hydrogen bonding between the carboxylic units and thus prevents the formation of β -sheet type network (shown on Figure 5).

Computational Analysis

In order to rationalize the propensity of gelator molecules to assemble and to evaluate the effect of Na^+ cations on the aggregation, we performed a computational analysis using the M06-2X DFT functional in the gas phase. In doing so, we utilized a model analog of the gelator molecule **1**, since we

demonstrated that it showed the best gelation properties, in which we replaced the isobutyl group by the methyl one to obtain **1a** as shown in Figure 7.

It turns out that in isolated gas-phase systems, both *trans*-**1a** stereoisomers are around $9\text{--}10 \text{ kcal mol}^{-1}$ more stable than their *cis*- counterparts, with **tG1** having the methyl groups on different sides of the plane, being the most stable system. This implies that *cis*-**1a** conformers are not favorable in the gas phase and their presence is very unlikely, being fully in line with experiments reported here.

Figure 7. Different conformers and stereoisomers of **1a** and their relative stabilities at the M06-2X/6-311++G(d,p)//M06-2X/6-31+G(d) level of theory (in kcal mol⁻¹)

We investigated the aggregation of **1a** molecules by sequentially adding individual monomers to the system to obtain the matching dimers, trimers, and tetramers. The calculated interaction free energies and schematic representation of the most stable structures are presented in Scheme 1, while the graphical representation of these aggregates is given in Supplementary Information Figure S7.

Dimerization of **1a** is favorable in the gas phase with the interaction free energy of -5.9 kcal mol⁻¹. The dimer is stabilized through two types of hydrogen bonds: (a) those between the central amide fragments, and (b) those among the carboxyl units on both sides of **1a**, which are persistent motifs also evident in higher aggregates (Scheme 1). In (tG1)₂ dimer, hydrogen bonds in amide fragments are associated with O–N distances of 2.96 Å, which appear to be out-dominated by the hydrogen bonds among carboxyl groups, where all four O–O distances are shorter, being either 2.70 or 2.74 Å, suggesting the latter provides a prevailing stabilizing factor in the dimer.

This behavior could be rationalized by inspecting the intrinsic acidity and basicity of these fragments in **1a**, since these are strongly related to their abilities to form and accept hydrogen bonds [36], respectively. In **1a**, the carboxylic group is by 5.5 kcal mol⁻¹ more O–H acidic and by 4.1 kcal mol⁻¹ more basic than the corresponding amide N–H and carbonyl moieties, respectively, which both indicate stronger propensity of the former fragment to participate in the formation of hydrogen bonding. This trend is continued in the trimer (tG1)₃, where the interacting O–N distances assume 2.80 and 3.06 Å, whereas the corresponding O–O distances are shorter and all are found at around 2.76 Å, the same being observable in the tetramer (tG1)₄ as well. This led us to conclude that the aggregation of **1a** molecules is primarily determined through the interaction of terminal carboxyl fragments, which allows favorable orientation for the hydrogen bonding among *trans* amide groups. Our attempts to model the intermolecular association of the carboxyl groups in tetramer (tG1)₄ as in Figure 5 gave a much less

Scheme 1. Calculated gas-phase interaction free energies for the aggregation of **1a** molecules at the M06-2X/6-311++G(d,p)//M06-2X/6-31+G(d) level of theory (all values in kcal mol⁻¹)

stable system, leading us to conclude that such organization may be evident in much higher aggregates or in the solid state as explained earlier. The obtained interaction energies imply that the gelator aggregation is thermodynamically a favorable process and suggest that it will occur spontaneously in the gas phase with every additional monomer contributing moderately to the overall stabilization. In concluding this section, let us mention that our attempts to model the aggregation of *cis-1a* molecules resulted in either systems much less stable than the corresponding *trans* aggregates or the monomeric units spontaneously rotated to *trans* conformers during the geometry optimization. For example, the most stable *cis-1a* dimer is 23.6 kcal mol⁻¹ less stable than its *trans-1a* analog.

Introduction of Na⁺ cations into the system changes conformational and binding properties of **1a** molecules. Interestingly, in **1a**:Na⁺ complex there is an inversion of the stability in **1a**, with the *cis-1a* conformation becoming 0.2 kcal mol⁻¹ more stable than the matching *trans-1a* due to the ability of the former to bind the Na⁺ cation in a tridentate fashion (Scheme 2). In *cis-1a*:Na⁺ complex, distances between carbonyl oxygens and the Na⁺ cation are 2.30 and 2.36 Å, with one carboxyl group also binding Na⁺ at 2.29 Å. A conformation with tetradentate Na⁺ binding in *cis-1a* is also possible, but is 3.9 kcal mol⁻¹ less stable. What is particularly interesting is that the interaction free energy in *cis-1a*:Na⁺ system is as high as

−39.3 kcal mol⁻¹, which is much higher than the energies calculated for the self-aggregation of **1a** molecules. This convincingly suggests that the presence of the Na⁺ cations easily disrupts the organization of **1a** systems already at the level of 1:1 complexes. If we investigate the ability of **1a** to bind two Na⁺ cations, it turns out that *trans-1a* complex becomes 9.1 kcal mol⁻¹ more stable due to the fact that both Na⁺ cations are bound on different sides of **1a** with Na–Na separation of 7.78 Å (Scheme 2). If the *cis* counterpart, both Na⁺ cations are placed much closer at 5.71 Å, which reduces the overall stability. Surprisingly, the calculated interaction free energy in 1:2 *trans-1a*:Na⁺ complex is −23.2 kcal mol⁻¹, which is lower than in the 1:1 complex. This implies that the binding of two Na⁺ cations to one **1a** molecule is less favorable than for one Na⁺ cation, demonstrating that the formation of 1:2 = **1a**:Na⁺ complex is very unlikely.

Binding of one Na⁺ cation to two **1a** molecules is even more favorable than in the corresponding 1:1 complexes. Complexation with *trans-1a* systems once again becomes more stable by 14.1 kcal mol⁻¹ (Scheme 2), with the interaction free energy being further increased from the 1:1 complex to −67.6 kcal mol⁻¹. This could be rationalized by the fact that 2:1 complex with *trans-1a* molecules, apart from the central tetra-coordination of the Na⁺ cation, allows for a significant hydrogen bonding among carboxyl groups between two gelators

Scheme 2. Calculated gas-phase interaction free energies for the complexation between **1a** molecules and Na⁺ cations at the M06-2X/6-311++G(d,p)//M06-2X/6-31+G(d) level of theory (all values in kcal mol⁻¹)

(Scheme 2), which is absent in both *cis*-**1a**. In other words, in 2:1 = *trans*-**1a**:Na⁺ complex, the corresponding O–O distances in the hydrogen bonded carboxyl groups are both 2.75 Å, being almost perfectly linear at 174.9°. This even allows shorter Na–O distances in the central part, being 2.22 and 2.26 Å, which suggest stronger binding than in *cis*-**1a** where these assume 2.29 Å.

A single Na⁺ cation can bind three **1a** molecules and this indeed occurs in the gas phase, again much more likely with *trans*-**1a**. In this case (Supplementary Information Fig. S7), each **1a** coordinates Na⁺ in a bidentate way fully analogous to that found in 1:1 complex, in other words with one carbonyl group and the neighboring carboxyl group. In doing so, all three **1a** molecules form an octahedron around the Na⁺ cation with the corresponding Na–O distances of 2.23 Å towards the carbonyl oxygens and 2.46 Å towards carboxyl oxygens. Although this proceeds without any significant intermolecular interaction among the rest of **1a** molecules, the calculated gas-phase interaction free energy is –71.9 kcal mol^{–1}, being the highest in all studied **1a**:Na⁺ complexes. This convincingly implies that the formation of the 3:1 = **1**:Na⁺ complex is very likely and thermodynamically favorable, and rationalizes the experimentally observed fact that the dominant signal in the MS spectra corresponds exactly to this system. It also demonstrates that the presence of the Na⁺ cations in the system will undoubtedly disrupt any self-organization or assembly of the individual **1a** molecules, since the electrostatic interactions with Na⁺ cations significantly outperform interactions among **1a**'s. This is fully in line with experimental findings presented here.

Conclusion

The idea was to explore the difference in self-organization in the gas phase as the indicator of molecular recognition interactions between the oxalamide building blocks and that the physical linking could be observed because of the non-covalent supramolecular interactions. We observed self-assembled aggregates formed because of the strong intermolecular hydrogen bonding even though the transition from condensed phase into the high vacuum of the mass spectrometer could change the properties of the non-covalent bonds such as strength, directionality, and geometry. Taking all of this into account, we believe that the observed results in the gas phase could be related to the gelation properties of presently studied four compounds. Namely, compound **1** formed large stable aggregates in all studied solutions. Compounds **2** and **3** are not as good gelators as **1**, and did not preserve self-aggregation in the gas phase. The addition of metal ions disrupts the gelator network supported with the hydrogen bonding, which is fully corroborated by DFT calculations. These computations revealed that the presence of the Na⁺ cations in the system disrupts any self-organization of the individual **1** molecules since the electrostatic interactions with Na⁺ cations significantly outperform interactions among **1**'s, being fully in line with experimental findings presented here.

Acknowledgements

The authors gratefully acknowledge Dr. J. Makarević, (Laboratory for Supramolecular Chemistry, Division of Organic Chemistry and Biochemistry, Ruđer Bošković Institute, Zagreb, Croatia) for aid in the synthesis of gelators and the preparation of gels samples. This work was supported by the Ministry of Science, Education, and Sports of the Republic of Croatia under grant number 098-0982914-2935. R.V. gratefully acknowledges the financial support from the Croatian Science Foundation (HRZZ) under grant number IP-2014-09-3386 and L.F. under grant number IP-11-2013-7387.

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